REMARKS/ARGUMENTS

Claims 42 and 44-75 are pending in the above-referenced patent application and are currently under examination. With entry of this Amendment, claims 42 and 69 have been amended to more explicitly recite that the nucleic acid is encapsulated in the lipid of the particle and is resistant in aqueous solution to degradation with a nuclease. Support for the amendment to the claims can be found throughout the specification as originally filed (*see, e.g.*, page 3, line 28 through page 4, line 7, page 23, lines 13-20 and lines 29-32, page 24, lines 11-14, page 26, lines 21-24, the Examples, Figure 3, *etc.*) and, thus, no new matter has been introduced. In addition, Example 7 has been amended to correctly recite the following lipid composition: DOPE:DODAC:PEG-Cer-C_{20}. Support for the amendment to the specification can be found in Figure 15 as originally filed and, thus, no new matter has been introduced.

In the Office Action, claims 42 and 44-61 and 63-75 remain rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Choi *et al.* (U.S. Patent No. 5,820,873). In addition, claims 42 and 44-61, 63-75 have been newly rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Holland *et al.* (U.S. Patent No. 5,885,613). Finally, claim 62 has been newly rejected under 35 U.S.C. § 103 as allegedly being obvious over Choi *et al.* For the reasons set forth herein, each of the foregoing rejections is overcome.

The Invention

Novel nucleic acid-lipid particles that are useful for *in vitro* or *in vivo* gene transfer are provided by the present invention. The particles can be formed using either detergent dialysis methods or methods that utilize organic solvents. Upon removal of a solubilizing component (*i.e.*, the detergent or the organic solvent), the nucleic acid-lipid complexes form particles, wherein the nucleic acid is encapsulated in the lipid, is serum-stable and is protected from nuclease degradation.

Rejection Under 35 U.S.C. § 102(e) Over Choi et al.

Claims 42 and 44-75 remain rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5, 820,873 ("Choi *et al.*"). In view of the following remarks, Applicants respectfully traverse the rejection.

> To anticipate a claim, a reference must disclose each and every element of the challenged claim and must enable one skilled in the art to make the anticipated subject matter.

See, *PPG Industries Inc. v.* Guardian *Industries Corp.*, 37 USPQ2d.

In order to expedite prosecution of the present case, claim 42 has been amended to recite: "a nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a nucleic acid, wherein the nucleic acid ***is encapsulated in the lipid*** of said particle and is resistant in aqueous solution to degradation with a nuclease. In addition, claim 69 has been amended to recite: "a pharmaceutical composition comprising a nucleic acid-lipid particle and a pharmaceutically acceptable carrier, said nucleic acid-lipid particle comprising a cationic lipid, a conjugated lipid that inhibits aggregation of particles, and a nucleic acid, wherein said nucleic acid is encapsulated in the lipid of said particle and is resistant in aqueous solution to degradation with a nuclease.

As stated on page 4, lines 29-31, of the specification, the nucleic acid-lipid particles of the present invention are constructed in a way such that upon removal of a solubilizing component (*i.e.*, the detergent or the organic solvent depending on which methods is employed), the ***nucleic acid is encapsulated in the lipid and becomes protected from nuclease degradation***. The nucleic acid-lipid particles thus formed are suitable, *inter alia*, for use in intravenous nucleic acid transfer as they are stable in circulation, of a size required for pharmacodynamic behavior resulting in access to extravascular sites, and target cell populations.

As previously explained, Choi *et al.* teach a novel class of polyethylene glycol modified ceramide lipids, *i.e.*, PEG-ceramide conjugates, that can be used to form liposomes and other lipid formulations containing various biological agents or drugs.

In citing Choi *et al.*, the Examiner cites to (1) column 13, lines 35-44, as teaching that the PEG-ceramide conjugates of Choi *et al.* can be used in the formation of liposome

structures incorporating or entrapping one of more bioactive agents ; (2) Example 9 as teaching that the liposomes prepared according to the teachings of Choi *et al.* are capable of entrapping a bioactive agent; and (3) column 17, line 66 through column 18, line 24 as teaching that the bioactive agent can be a nucleic acid (*see*, pages 3 and 5 of the Office Action).

Applicants agree with the Examiner that the specification of Choi *et al.* discloses that a lipid of Formula I, *i.e.*, the PEG-ceramide conjugates, can be utilized in liposome structures incorporating or entrapping one or more bioactive agents. Applicants also agree with the Examiner that the specification of Choi *et al.* discloses that the bioactive agent can be a nucleic acid. Applicants further agree with the Examiner that Example 9 discloses a method for loading the bioactive agent vincristine into liposomes containing a lipid of Formula I. However, Applicants ***disagree*** with the Examiner that the specification of Choi *et al.* teaches that the encapsulation/loading method of Example 9 can be used to encapsulate or load nucleic acids into liposomes.

A perusal of the Choi *et al.* specification reveals that the teachings with respect to the bioactive agent being a nucleic acid are very specific and are directed to the formation of nucleic acid-lipid complexes for the delivery of such nucleic acids (*see*, column 17, line 64 through column 18, line 23). More particularly, in the specification, Choi *et al.* state:

> Cationic lipids may be used in the delivery of therapeutic genes or oligonucleotides intended to induce or to block production of some proteins within the cell. ***Nucleic acid is negatively charged and must be combined with a positively charged entity to form a lipid complex suitable for formation and cellular delivery.***

See, column 17, line 66 through column 18, line 4 (emphasis added). Clearly, this teaching of Choi *et al.* is directed to the formation of a ***nucleic acid-cationic lipid complex*** for the delivery of nucleic acid.

Moreover, the Choi *et al.* specification references two articles that are directed to the preparation and use of nucleic acid-liposome complexes. In both of these references, preformed liposomes are complexed with nucleic acids to form nucleic acid-liposome complexes, *i.e.*, lipoplexes. In the Wang *et al.* reference, liposomes comprising DOPE/CHOL/OA or

DOPC/CHOL/OA are formed and mixed with nucleic acid (*see*, column 1 of page 7852 of Wang *et al.*, a copy of which is attached as Exhibit A for the convenience of the Examiner). Similarly, in the Hyde *et al.* reference, cationic liposomes are formed and then complexed with plasmid DNA (*see*, column 1 of page 251 of Hyde *et al.*, a copy of which is attached as Exhibit B for the convenience of the Examiner). Clearly, such teachings of Choi *et al.* are directed to the formation of ***nucleic acid-lipid complexes*** for the delivery of nucleic acids.

> The Choi *et al.* specification further states:
>
> The efficiency of this transfection has often been less than desirable, for various reasons. ***One is the tendency for cationic lipids complexed with nucleic acids to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.***

See, column 18, lines 12-16 (emphasis added). Clearly, this teaching of Choi *et al.* is directed to improving ***nucleic acid-lipid complexes*** for delivery of nucleic acids by the inclusion of PEG lipids, such as the PEG-ceramide conjugates of Formula I.

> Finally, the Choi et *al.* specification states:
>
> Cationic lipids useful in producing lipid-based carriers for gene and oligonucleotide delivery are LIPOFECTIN...and LIPOFECTASE...Both agents, as well as other transfecting cationic lipids, are available from Life Technologies, Inc. in Gaithersburg, Md.

See, column 18, lines 17-23. Clearly, this teaching of Choi *et al.* is directed to examples of cationic lipids that can be used to form ***nucleic acid-cationic lipid complexes*** for the delivery of nucleic acids.

In addition to the foregoing, Example 11 of Choi *et al.* demonstrates that aggregation of the cationic liposomes alone (no DNA) can be inhibited in the presence of serum (most serum proteins carry a net negative charge) if the liposomes contain a PEG-ceramide conjugate. This example is consistent with the teachings set forth above directed to the formation of nucleic acid-cationic lipid complexes and the benefits that can be achieved if PEG lipids are incorporated into such complexes.

As such, the specification of Choi *et al.* teaches that if the bioactive agent is a nucleic acid, the nucleic acid is complexed with a cationic liposome comprising a lipid of Formula I, *i.e.*, a PEG-ceramide conjugate. Again, the specification of Choi *et al.* does ***not*** teach or suggest that the encapsulation method set forth in Example 9 can be used to encapsulate or load nucleic acid into a liposome.

As explained by both Michael J. Hope, Ph.D. and Sean Semple, M.Sc. in their previously filed declarations (hereinafter, "the Hope Declaration" and "the Semple Declaration," respectively), the nucleic acid-catioinc liposome complexes of Choi *et al.* are ill-defined, are only partially protected from nucleases, are heterogeneous in size and are rapidly cleared from the circulation. In contrast to such nucleic acid-cationic liposome complexes, the present invention provides novel methods by which nucleic acids (*e.g.*, oligonucleotides, plasmid DNA, *etc.*) are entrapped, *i.e.*, encapsulated, within cationic liposomes that include a conjugated lipid, such as a PEG-lipid conjugate. As explained in the specification and as set forth in the presently pending claims, the PEG-lipid conjugate prevents aggregation of the particles during formation, thereby resulting in nucleic acid-lipid particles of a homogeneous and defined size containing nucleic acid that is fully encapsulated in the lipid bilayer such that the nucleic acid is completely protected from nuclease degradation. According to the Semple Declaration, this is in stark contrast to the complexes or lipoplexes that would be formed based on the cationic liposomes of Choi *et al.*, which contain PEG-ceramide conjugates.

In support of this position, the Semple Declaration points to Figure 2 of Wheeler, *et al.*, *Gene Therapy*, 6:271-281 (1999); and Figure 1 of Monck *et al.*, *J. Drug Targeting*, 7:(6):439-452 (2000), copies of which were attached to the Semple Declaration as Exhibits B and C. In further support of this position, Applicants point the Examiner to Mauer *et al.*, *Mol. Membr. Biol.*, 16:129-140 (1999), a copy of which is attached hereto as Exhibit B, which further points to the differences between nucleic acid-cationic liposome complexes, such as those disclosed in Choi *et al.*, and the presently claimed nucleic acid-lipid particles, which are referred to therein as "SPLP."

Moreover, the specification of the present patent application explicitly points out the differences between the prior art complexes and the nucleic acid-lipid particles of the present invention, when it states:

> Lipid-nucleic acid formulations can be formed by combining nucleic acid with a preformed cationic liposome (see, U.S. Patent Nos. 4,897,355, 5,264,618, 5,279,833 and 5,283,185). In such methods, the nucleic acid is attracted to the cationic surface charge of the liposome and the resulting complexes are thought to be of the liposome-covered "sandwich-type." ***As a result, a portion of the nucleic acid or plasmid remains exposed in serum and can be degraded by enzymes such as DNAse I.*** Others have attempted to incorporate the nucleic acid or plasmid into the interior of a liposome during formation. These methods typically result in the aggregation in solution of the cationic lipid-nucleic acid complexes (Figure 2). Passive loading of a plasmid into a preformed liposome has also not proven successful. Finally, the liposome-plasmid complexes which have been formed are typically 200 to 400 nm in size and are therefore cleared more rapidly from circulation than smaller complexes or particles.

See, page 23, lines 13-20 of the specification of the present patent application (emphasis added).

In view of the foregoing remarks, the previously submitted Hope Declaration and the previously submitted Semple Declaration, Choi *et al.*, which discloses methods for preparing and loading classical (or traditional) liposomes and methods for preparing nucleic acid-cationic liposome complexes, do ***not*** teach the nucleic acid-lipid particles of the present invention, wherein the nucleic acid in the nucleic acid-lipid particles is ***encapsulated in the lipid*** and is ***resistant in aqueous solution to degradation with a nuclease*** or methods for making such nucleic acid-lipid particles. Because Choi *et al.* do ***not*** disclose each and every element of the claimed invention, it cannot form the basis of a proper anticipation rejection. Accordingly, the anticipation rejection under 35 U.S.C. § 102(e) over Choi *et al.* is improper and should be withdrawn.

Rejection Under 35 U.S.C. § 102(e) Over Holland et al.

Claims 42 and 44-75 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by U.S. Patent No. 5,885,613 ("Holland *et al.*"). In view of the following remarks, Applicants respectfully traverse the rejection.

A perusal of Holland *et al.* reveals that the teachings provided therein directed to the delivery of nucleic acids are essentially the same as those provided in Choi *et al.* More particularly, Holland *et al.* states:

> For the delivery of therapeutic agents, the fusogenic liposomes of the present invention can be loaded with a therapeutic agent and administered to the subject requiring treatment. The therapeutic agents which can be administered using the fusogenic liposomes of the present invention can be any of a variety of drugs, peptides, proteins, DNA, RNA or other bioactive molecules. Moreover, cationic lipids may be used in the delivery of therapeutic genes or oligonucleotides intended to induce or to block production of some protein within the cell. ***Nucleic acid is negatively charged and must be combined with a positively charged entity to form a complex suitable for formulation and cellular delivery.***

See, column 12, lines 14-26 of Holland *et al.* (emphasis added).

Moreover, Holland *et al.* state:

> Cationic lipids have been used in the transfection of cells in vitro and in vivo. . . .The efficiency of this transfection has often been less than desired, for various reasons. ***One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.***

See, column 12, lines 14-26 of Holland *et al.* (emphasis added).

Clearly, as with Choi *et al.*, the teachings of Holland *et al.* are directed to forming nucleic acid-cationic liposome complexes, which are structurally and functionally different from the presently claimed nucleic acid-lipid particles, wherein the nucleic acid component is encapsulated in the lipid component and is resistant in aqueous solution to degradation with a nuclease.

As such, for the reasons set forth above in connection with the § 102(e) rejection over Choi *et al.*, Applicants respectfully submit that Holland *et al.*, which discloses the ***same*** methods as Choi *et al.* for preparing and loading classical (or traditional) liposomes and for preparing nucleic acid-cationic liposome complexes, do ***not*** teach the nucleic acid-lipid particles of the present invention, wherein the nucleic acid in the nucleic acid-lipid particles is ***encapsulated in the lipid*** and is ***resistant in aqueous solution to degradation with a nuclease*** or methods for making such nucleic acid-lipid particles. Because Holland *et al.* do ***not*** disclose each and every element of the claimed invention, it cannot form the basis of a proper anticipation rejection. Accordingly, the anticipation rejection under 35 U.S.C. § 102(e) over Holland *et al.* is improper and should be withdrawn.

Rejection Under 35 U.S.C. § 103(a) Over Choi et al.

Claim 62 is rejected under 35 U.S.C. § 103(a) as allegedly being obvious over U.S. Patent No. 5, 820,873 ("Choi *et al.*"). In view of the following remarks, Applicants respectfully traverse the rejection.

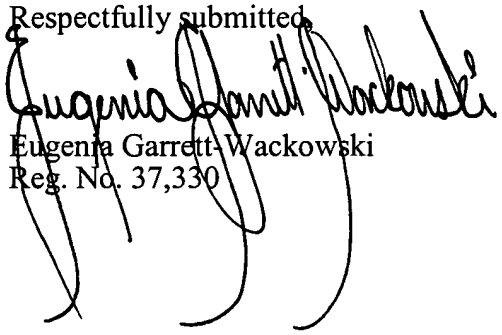
As explained above in connection with the § 102(e) rejection, Choi *et al.* do ***not*** teach (or suggest) the nucleic acid-lipid particles of the present invention, wherein the nucleic acid in the nucleic acid-lipid particles is ***encapsulated in the lipid*** and is ***resistant in aqueous solution to degradation with a nuclease*** or methods for making such nucleic acid-lipid particles. Because Choi *et al.* do ***not*** teach or suggest the presently claimed nucleic acid-lipid particles, Choi *et al.* do not teach or suggest the nucleic acid-lipid particles of claim 42, wherein the nucleic acid is a ribozyme. Accordingly, the obviousness rejection under 35 U.S.C. § 103(a) over Choi *et al.* is improper and should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,



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